In Vitro Skin Corrosivity Methods: EPISKIN™, EpiDerm™ (EPI-200), and the Rat Skin Transcutaneous Electrical Resistance (TER) Assay

Executive Summary

Corrosive substances are defined as chemicals causing visible destruction or irreversible alterations in living tissue by chemical action at the site of contact (29 CFR 1917.28). Dermal corrosivity testing is conducted to identify chemicals that potentially pose this hazard to humans. U.S. Federal regulations and guidelines include test methods for assessing dermal corrosivity. Testing data is used to determine appropriate hazard classification and labeling, appropriate transport and/or storage methods, and appropriate precautions for workers in industrial settings. For regulatory purposes, corrosive chemicals and chemical mixtures have typically been identified using an *in vivo* test method involving the application of chemicals or chemical mixtures to the intact skin of a rabbit. The skin is visually evaluated for corrosion after exposures of three minutes, one hour, and four hours. Animal welfare considerations have led to efforts to develop *in vitro* alternative test methods. One such method, Corrositex□, (In Vitro International, Inc., Irvine, CA) was submitted to ICCVAM for consideration. Following independent scientific peer review (ICCVAM, 1999), ICCVAM recommended that Corrositex□ could be used to assess the dermal corrosion potential of chemicals as part of a tiered testing strategy.

Three other alternative *in vitro* test methods – EpiDerm™ (EPI-200), EPISKIN™, and the Rat Skin Transcutaneous Electrical Resistance (TER) assay – have subsequently undergone validation studies by the European Centre for the Validation of Alternative Methods (ECVAM), and have been accepted for corrosivity testing in the European Union (EU, 2000). ICCVAM subsequently implemented an expedited test method review process to consider methods which have been evaluated by the ECVAM (ICCVAM, 2001). This process will accelerate interagency consideration of these test methods, thereby avoiding duplication of effort and unnecessary delays in recommending useful test methods to Federal agencies in accordance with Public Law 106-545. This report describes the information and data considered by ICCVAM during its expedited review of the three methods, and provides the ICCVAM test recommendations for these methods.

Validation and Regulatory Acceptance Status of EPISKIN™, EpiDerm™ (EPI-200), and the Rat Skin TER Assay

Independent validation studies on these three *in vitro* assays were conducted by ECVAM (Barratt et al., 1998; Fentem et al., 1998; Liebsch et al., 2000). The ECVAM Validation Management Team concluded that the EpiDerm™ (EPI-200), Rat Skin TER, and EPISKIN™ methods were able to distinguish between corrosive and non-corrosive chemicals for all of the chemical classes considered.

A review of these validation studies and the analyses conducted by NICEATM are presented in Sections 2.0, 3.0, and 4.0 of this report.

The validation status of the three methods was reviewed by the ECVAM Scientific Advisory Committee (ESAC) (Balls and Corcelle, 1998; Balls and Hellsten, 2000). The ESAC

concluded that the Rat Skin TER, Episkin□, and EpiDerm™ (EPI-200) tests can be used to distinguish between corrosive and non-corrosive chemicals within the context of the draft EU and Organisation for Economic Co-operation and Development (OECD) test guidelines on skin corrosion (Balls and Corcelle, 1998; Balls and Hellsten, 2000). EPISKIN□ and Rat Skin TER were also reviewed by the European Commission's Scientific Committee for Cosmetic Products and Non-food Products (SCCNFP) which concluded that the methods were considered applicable to the safety evaluation of cosmetic ingredients or mixtures of ingredients (SCCNFP, 1999).

EPISKIN™

The EPISKIN□ human skin model is commercially available from EPISKIN SNC, Lyon, France, a wholly owned subsidiary of L'OREAL. EPISKIN™ is a three-dimensional human skin model composed of a human collagen (Types III and I) matrix, representing the dermis, covered with a film of Type IV human collagen and stratified differentiated epidermis derived from human keratinocytes. Test materials can be applied directly to the stratum corneum. The model utilizes cell viability as the measured endpoint. The topical mode of application of test material mimics the route of human exposure. For use in corrosivity testing, the test material (liquids: 50 µL; solids: 20 mg) is applied to an epidermis unit for 3, 60, and 240 minutes. Cell viability is assessed by measuring mitochondrial activity using the MTT (a tetrazolium salt) assay as compared to concurrent negative controls. A 35% decrease in cell viability is used to indicate a potential for human corrosivity.

ECVAM conducted an independent validation study on the EPISKIN™ method as an *in vitro* replacement assay for *in vivo* corrosivity testing (Fentem et al., 1998). Sixty chemicals were evaluated in duplicate in three different laboratories; chemical selection and *in vivo* reference data were described by Barratt et al. (1998). The ECVAM validation chemical test set included:

- organic acids
- organic bases
- neutral organics
- phenols
- inorganic acids
- inorganic bases
- inorganic salts
- electrophiles
- soaps/surfactants

The database used in the EPISKIN™ evaluation consisted of data from the ECVAM validation study only; other data were not located. An analysis of the results of the database of 60 chemicals and chemical mixtures evaluated in the validation study had the following performance:

- accuracy: 83% (50/60 chemicals or chemical mixtures)
- sensitivity: 82% (23/28 chemicals or chemical mixtures)
- specificity: 84% (27/32 chemicals or chemical mixtures)
- false positive rate: 16% (5/32)
- false negative rate: 18% (5/28)

Furthermore, EPISKIN™ was able to distinguish between known R35/I and R34/II & III chemicals.[1]

Inter- and intra-laboratory reproducibility of EPISKIN™ was also evaluated by Fentem et al. (1998). In each laboratory, each chemical was tested three times using three different batches of EPISKIN™. Of the 60 chemicals tested, 42 gave the same corrosivity classification in all three tests in all three laboratories. Discordant results for the remaining chemicals were as follows: one of nine tests for six chemicals, two to three of nine tests for seven chemicals, and four to five of nine tests for the remaining five chemicals. The study concluded that EPISKIN™ had acceptable intra- and inter-laboratory reproducibility (Fentem et al., 1998).

EpiDerm™ (EPI-200)

EpiDerm™ (EPI-200) is commercially available from MatTek Corporation, Ashland, MA, USA. The EpiDerm™ (EPI-200) skin model is mechanistically and functionally related to EPISKIN™. The assay consists of normal human epidermal keratinocytes which have been cultured in chemically defined medium to produce a stratified, highly differentiated, organotypic tissue model of the human epidermis. The EpiDerm™ (EPI-200) tissue consists of metabolically and mitotically active cells which are organized into basal, spinous, and granular layers along a multi-layered stratum corneum (MatTek Corporation, 2000). Like EPISKIN™, the EpiDerm™ (EPI-200) tissue approximates the barrier of normal human skin, and the topical mode of application of the test material in EpiDerm™ (EPI-200) mimics the route of human exposure. For use in corrosivity testing, the test material (liquids and semi-solids: 50 µL; solids: 25 mg plus 25 µl of H2O) is applied to a tissue for three and 60 minutes. For each test substance, duplicate plates are analyzed at each test period. As with EPISKIN™, cell viability is assessed by measuring mitochondrial activity using the MTT assay. A test chemical is classified as corrosive if it induces ≥50% decrease in relative cell viability at 3 minutes or ≥85% decrease in relative cell viability at 60 minutes.

ECVAM conducted an independent validation study on EpiDerm™ (EPI-200) as an *in vitro* replacement assay for *in vivo* corrosivity testing (Liebsch et al., 2000). Twenty-four chemicals representative of the 60 chemicals tested in the Fentem et al. (1998) ECVAM validation study for the EPISKIN™ assay were tested. The 24 chemicals selected included 12 corrosive and 12 noncorrosive chemicals composed of; organic acids and bases, neutral organic bases, phenols, inorganic acids and bases, electrophiles, and surfactants.

The database used in the evaluation of EpiDerm™ (EPI-200) consisted of data from the ECVAM pre-validation/validation study only (Liebsch et al., 2000); other data were not located. (see Section 2.0) Based on an analysis of the results of 24 chemicals and chemical mixtures evaluated in the validation study, EpiDerm™ (EPI-200) had the following performance:

[1]UN packing group classifications I, II, and III are assigned based on the capacity of a chemical, when tested on the intact skin of rabbits, to produce skin corrosion following exposure intervals of 3 minutes, 1 hour, or 4 hours, respectively (Fentem et al., 1998). EU regulations require classification of chemicals according to certain risk phases, such as those assigned based on whether the chemical causes corrosion following a 3-minute application (R35 – "causes severe burns"; analogous to packing group I) or 4 hours (R34 – "causes burns"; analogous to packing groups II and III) (Barratt et al., 1998; Fentem et al., 1998).

- accuracy: 92% (22/24 chemicals or chemical mixtures)
- sensitivity: 92% (11/12 chemicals or chemical mixtures)
- specificity: 83% (10/12 chemicals or chemical mixtures).
- false positive rate: 17% (2/12)
- false negative rate: 8% (1/12)

Unlike EPISKIN™, EpiDerm™ (EPI-200) was not able to distinguish between known R35/I and R34/II & III chemicals.

Intra- and inter-laboratory reliability was evaluated by testing each chemical twice, using different tissue lots, in each of three laboratories. Of the 24 chemicals tested, 19 gave the same corrosivity classification in the two replicates in all three laboratories (six tests). Discordant results for the remaining chemicals were as follows: one of six tests for three chemicals and two of six tests for two chemicals. Based on the results obtained, the study concluded that EpiDerm™ (EPI-200) provided excellent reliability (Liebsch et al., 2000).

Rat Skin TER

Transcutaneous electrical resistance is measured using an AIM electronic databridge 401 or 6401, which is commercially available from H. Tinsley and Co., New Addington, Croydon, Surrey, UK. In the Rat Skin TER assay, test materials (liquids: 150 µL; solids 100 mg plus 150 µL of water) are applied for two and 24 hours to the epidermal surfaces of skin discs obtained from the skin of humanely killed young rats. Nine to 15 discs can be prepared from one rat pelt which can be used to test up to five chemicals. Corrosive materials produce a loss of normal stratum corneum integrity and barrier function, which is measured as a reduction of the inherent transcutaneous electrical resistance below a predetermined threshold level of 5 kΩ.

A prevalidation study of the Rat Skin TER assay was conducted during 1993 and 1994 (Botham et al., 1995) to evaluate the relative performance and interlaboratory variability of the method. Subsequently, in 1997, the Rat Skin TER method was also evaluated in an ECVAM validation study as an alternative for traditional *in vivo* testing using the same 60 chemicals and chemical mixtures as EPISKIN™ (Fentem et al., 1998).

The database used in the TER evaluation consisted of data from three published sources (Botham et al., 1992; Botham et al., 1995; Fentem et al., 1998). Based on a database of 122 chemical and chemical mixtures, TER had the following performance:

- accuracy: 81% (99/122 chemicals or chemical mixtures)
- sensitivity: 94% (51/54 chemicals or chemical mixtures)
- specificity: of 71% (48/68 chemicals or chemical mixtures)
- false positive rate: 29% (20/68)
- false negative rate: 6% (3/54)

These performance characteristics were not different when the Botham et al. (1992) and (1995) studies were evaluated independently of the ECVAM validation study (Fentem et al., 1998). The Rat Skin TER assay was not capable of classifying chemicals or chemical mixtures by UN corrosivity packing group.

In the ECVAM validation study (Fentem et al., 1998), the intra- and inter- laboratory reliability was evaluated. Inter- and intra-laboratory reproducibility were approximately equivalent, with no evidence of systematic differences between experiments within a laboratory. Of the 60 chemicals tested, 37 gave the same corrosivity classification in both experiments in all three laboratories (six

tests). Discordant results for the remaining chemicals were as follows: one of six tests for 11 chemicals and two to three of six tests for 12 chemicals. ECVAM concluded the TER assay had acceptable reproducibility.

ICCVAM Recommendations

Draft proposed test recommendations were developed by the ICCVAM Corrosivity Working Group (CWG), which was composed of Federal Agency scientists who have experience and/or expertise with corrosivity testing. These proposed recommendations were endorsed by ICCVAM and made available with background review materials for a 45-day public comment period as announced in a September 28, 2001, *Federal Register* notice (Appendix D).

Twenty-one public comments were received and considered by the CWG, which then drafted final test recommendations that were forwarded with the public comments for consideration by ICCVAM. The ICCVAM revised and approved the final test recommendations in May, 2002.

Based on an evaluation of the ECVAM validation studies and all other available data, ICCVAM concludes that there are sufficient data to substantiate the use of these assays for assessing the dermal corrosion potential of chemicals in a weight-of-evidence approach in an integrated testing scheme (OECD, 2001b; OECD, 2001d). EPISKIN™, EpiDerm™ (EPI-200), and Rat Skin TER are not appropriate methods for assessing irritation. The integrated testing schemes for dermal irritation/corrosion allow for the use of validated and accepted *in vitro* methods. In this approach, positive *in vitro* corrosivity responses do not generally require further testing and can be used for classification and labeling. Negative *in vitro* corrosivity responses would be followed by *in vivo* dermal irritation/corrosion testing. (Note: The first animal used in the irritation/corrosivity assessment would be expected to identify any chemical corrosives that were false negatives in the *in vitro* test). Furthermore, as is appropriate for any *in vitro* assay, there is the opportunity for confirmatory testing if false positive results are indicated based on a weight-of-evidence evaluation of supplemental information, such as pH, structure-activity relationships (SAR), and other chemical and testing information.

ICCVAM concludes also that each of the three *in vitro* corrosivity methods sufficiently consider and incorporate, where scientifically feasible and applicable, the 3Rs of animal use alternatives (refinement, reduction, and replacement). When EpiDerm™ (EPI-200) and EPISKIN™ are used as part of the integrated testing strategy for corrosivity/irritation, there is a reduction in the number of animals required because positive results usually eliminate the need for animal testing, and when further testing in animals is determined to be necessary, only one animal could be required to identify a corrosive chemical (one animal is used if the *in vitro* test is negative). Compared to the rabbit corrosivity test, the Rat Skin TER method reduces the number of animals used because skin from only one rat may be used to test up to five chemicals. Similar to EpiDerm™ (EPI-200) and EPISKIN™, use of the Rat Skin TER assay as part of the integrated testing strategy for corrosivity/irritation reduces and refines the use of animals when negative *in vitro* results are obtained.

[This Page Intentionally Left Blank]